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Membrane-based plasma collection device for point-of-care diagnosis of HIV☆

Arman Nabatiyan*, Zaheer A. Parpia, Robert Elghanian, David M. Kelso

Center for Innovation in Global Health Technologies, Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, United States

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ABSTRACT

A major requirement for the development of point-of-care tests for the detection of disease analytes is the need to separate plasma from whole blood in an efficient and rapid manner. Furthermore, the separated plasma must be able to elute efficiently the analyte of interest and serve effectively as a physical matrix to deliver the equivalent of neat plasma for downstream diagnostic analysis. Additionally, many applications require the use of heat shock to liberate immunocomplexed antigen found in the collected plasma. A membrane-based filter method is reported for rapid and efficient collection of plasma from a whole blood sample that is compatible with heat shock. Using pediatric human immunodeficiency virus as an example, this device elutes 100% of the input p24 core antigen post-collection and enables heat shock of plasma samples identical to neat plasma treatment.

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1. Introduction

The need for point-of-care diagnosis is highlighted by the growing rates of infectious diseases such as human immunodeficiency virus (HIV), pneumonia and tuberculosis in the developing world, and influenza and sexually transmitted diseases in the developed world. Such infections necessitate on-site diagnosis of infected individuals particularly in high risk populations so as to not only administer effective treatment to affected patients, but also monitor ongoing prevention efforts, assess the efficacy of existing prevention programs and improve opportunities for early therapy and disease prevention among infected individuals. In many instances, such as acute HIV infection, the need to measure disease prevalence can also be simplified with point-of-care technology by circumventing the need for longitudinal follow-up. In this context, point-of-care tests can be used to discriminate recent infections from established infections by cross-sectional sampling or a single sampling within the prevalent population.

Current methods for blood collection involve carrying out a venipuncture, collecting blood in a vacuutainer tube, centrifuging the blood for ten or more minutes and extracting the plasma with a pipette. This protocol requires a trained phlebotomist to draw blood and requires expensive vacuutainer tubes and a sterilized hypodermic needle. It requires additional capital equipment such as a centrifuge to separate the plasma fraction from the blood cells, refrigerators to store the sample and electricity to operate the equipment.

An alternative to venipuncture is to collect peripheral blood by lancing the fingertip or, in the case of infants, the heel. This provides a more economical means for sample collection that does not require a trained phlebotomist to perform and provides an option for the uniform and systematic collection of large number of field specimens for diagnostic and surveillance purposes, especially in developing countries. Instead of collecting blood in a tube and centrifuging it to separate the plasma from the cells, a porous matrix such as a filter membrane can be used to collect the blood and yield the plasma to a second, absorptive membrane (Cassol et al., 1991, 1992a,b, 1996; Li et al., 2005; Steger et al., 1990).

Pediatric HIV infection is a prime example of a disease that can benefit immensely from the use of point-of-care blood collection and diagnostic technology. Of all children 15 years and younger, 90% of those living with HIV and 90% of the AIDS related deaths are in Sub-Saharan Africa (UNP, 2008). Similar to the prevalence of HIV infection, current practices in HIV diagnostic testing vary significantly depending on world geography. In high-income countries such as the United States and Western Europe there is ready access to advanced HIV antibody/antigen combination immunoassays in addition to nucleic acid based assays (Chou et al., 2005; Read, 2007). In low-income countries such as those in Sub-Saharan Africa, access to later generation immunoassays and nucleic acid test assays is limited.

Thus for many resource limited settings affected with HIV, rapid self-performing tests for HIV antibodies are used because they

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* Corresponding author. Tel.: +1 847 467 0522; fax: +1 847 491 5299.
E-mail address: Arman@Northwestern.edu (A. Nabatiyan).

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address most of the technological and cost limitations imposed by nucleic acid based tests (Chou et al., 2005; De Baets et al., 2005; Prendergast et al., 2007; Read, 2007). While antibody tests are used commonly to diagnose HIV infection in adults, the persistence of passively transferred maternal antibodies make rapid tests inaccurate for infants (Bryson et al., 1995). HIV negative infants can be seropositive due to the transfer of maternal HIV antibodies. Consequently, detection of HIV in infants requires targeting the HIV core protein p24 as the principal marker for detection in order to verify unequivocally their true infection state irrespective of the maternal sero-inheritance. As maternally inherited antibodies in infants mask the detection of the HIV core p24 antigen, immune complex disruption (ICD) methods must be employed to liberate antigen for detection. The most effective means of ICD is achieved by heat shock of plasma in a buffer containing detergent (Schupbach et al., 2006). This presents an additional challenge for the development of a point-of-care infant diagnostic as the collected plasma must be amenable to heat shock.

This study reports on the development of a prototype unit compatible for use with an infant HIV antigen assay that combines blood collection and plasma separation to deliver a robust and simple method for plasma collection. This device provides a matrix to collect peripheral blood and separate cells from plasma in a self-performing manner. Thus the reported device is passive and does not require any active pumping or electricity to operate. Since the plasma is contained in a membrane at all times, this method also affords compatibility with heat shocking of a plasma sample without any form of liquid handling or loss of analyte. Lastly, as the plasma collection component of the design is sealed off from the environment, it allows for short-term storage of the plasma specimen and limited exposure of the test operator to potentially biohazardous material.

2. Materials and methods

2.1. Plasma separation unit

Vivid GF grade membrane (Pall Corporation; Ann Arbor, MI) was dip-coated with blocking solution (0.1% BSA/0.5% Sucrose/0.1% Tween-20 in water) to increase membrane hydrophilicity and blood sample flow over the surface. After dip-coating, membranes were dried for 20 min at 50 °C and then cut into 15.6 mm discs using a laser cutter (model X2-660, Universal Laser Systems) set at 25% power and 90% speed. The following plasma collection pads were cut in circular discs using a laser cutter set at 40% power and 100% speed: Grade 111, 121, 142, 151 (Ahlstrom; Mount Hollysprings, PA), Accuwik, Leukosorb B, A/B, A/D, A/E, Type III (Pall Corporation; Ann Arbor, MI), Fusion5 and 902 paper (Whatman; Florham Park, NJ).

Plasma separation was carried out using machined polypropylene parts that accommodated a range of plasma collection pads (up to 12 mm in diameter) on an insert and the Vivid blood separation membrane on the lip of the outer module, using a third plastic collar to hold the blood separation membrane in place on the lip via friction fit. The plasma collection pads were positioned with a screw mechanism so as to be flush with the blood separation membrane. After complete assembly of the device and components, 75 μL of whole blood was applied to the top of the blood separation membrane and separation allowed to proceed for 3–5 min until the blood had completely flowed through the Vivid membrane. To remove the plasma separation pad, the plastic collar was removed and the Vivid membrane lifted up with tweezers to expose the plasma collection pad that was also removed with tweezers and subjected to the lateral flow assay.

2.2. Lateral flow assay and test strips

Lateral flow test strips were generated and configured precisely as described elsewhere (Parpia et al., 2010). To run the assay using neat plasma, 25 μL plasma specimen was mixed with 75 μL heat shock buffer (0.67% NP-40 and 0.2% SDS in PBS) in 2 mL polypropylene tubes and heated for 4 min at 88 °C in a water bath. To run the assay using plasma collected in a pad, the pad containing adsorbed plasma specimen was put into 2 mL polypropylene tubes containing 75 μL heat shock buffer (0.67% NP-40 and 0.2% SDS in PBS), mixed with finger flicking and heated for 4 min at 88 °C in a water bath. After allowing the sample to cool to room temperature (~25 °C), an assay strip was inserted into the tube thereby initiating flow of sample into the capture membrane. In the case of reactions involving a plasma collection pad, direct contact was made between the collection pad and the capture membrane of the test strip containing dried down assay reagents. After 30 min, the assay result was scanned for quantitation.

2.3. Assay signal quantitation

The wet assay strips were scanned with a Canon flatbed scanner (model: Canoscan 3000EX) from which semi-quantitative measurements of p24 antigen concentration were obtained using a MatLab program. To obtain these measurements, an automated program located the control line for each strip, from where it scanned the area 5 mm upstream reporting the average intensity values in blocks of five pixel rows parallel to the control line. From this intensity profile, it calculated the background subtracted intensity (BSI) by subtracting the average intensity 1 mm upstream of the test line area. The highest BSI located between 1.5 and 5 mm was reported as the signal intensity value in arbitrary absorbance units for that strip.

2.4. Specimens

Heel-prick derived fresh infant blood was obtained daily from the central testing facility of Children's Memorial Hospital in Chicago, IL. Virology Quality Assurance (VQA) HIV was obtained from Dr. James Bremer (contract #NO1-AI-50044) at the VQA Laboratory at Rush St. Luke's Medical Center, Chicago, IL. The VQA virus control standard (subtype B) was received as viral culture spiked in normal human plasma at a concentration of 1.5 million copies/mL determined in house by the VQA laboratories (Jackson et al., 1993). Recombinant p24 protein was kindly provided by Dr. John Hackett at Abbott Diagnostics (Abbott Park, IL) at a stock concentration of 280 μg/ml. The protein concentration was determined using an Abbott HIV-1 p24 microtiter EIA. Briefly, an Abbott HIV-1 p24 Primary Standard was used to establish a standard curve to calculate p24 concentration. The Abbott p24 Primary Standard is calibrated to the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS) HIV-1 p24 standard.

3. Results

3.1. Development of the plasma separation module

Plasma separation modules were developed that could accommodate a membrane for blood separation and a second separate pad for plasma collection. These modules were machined out of polypropylene and integrated a screw lock mechanism which allowed for accurate positioning of the membrane and collection pad with respect to each other (Fig. 1A and B). In order to prevent either the blood separation membrane or collection pad from being compromised by shearing as the collection module is screwed towards the separation membrane, an anti-rotation pad platform

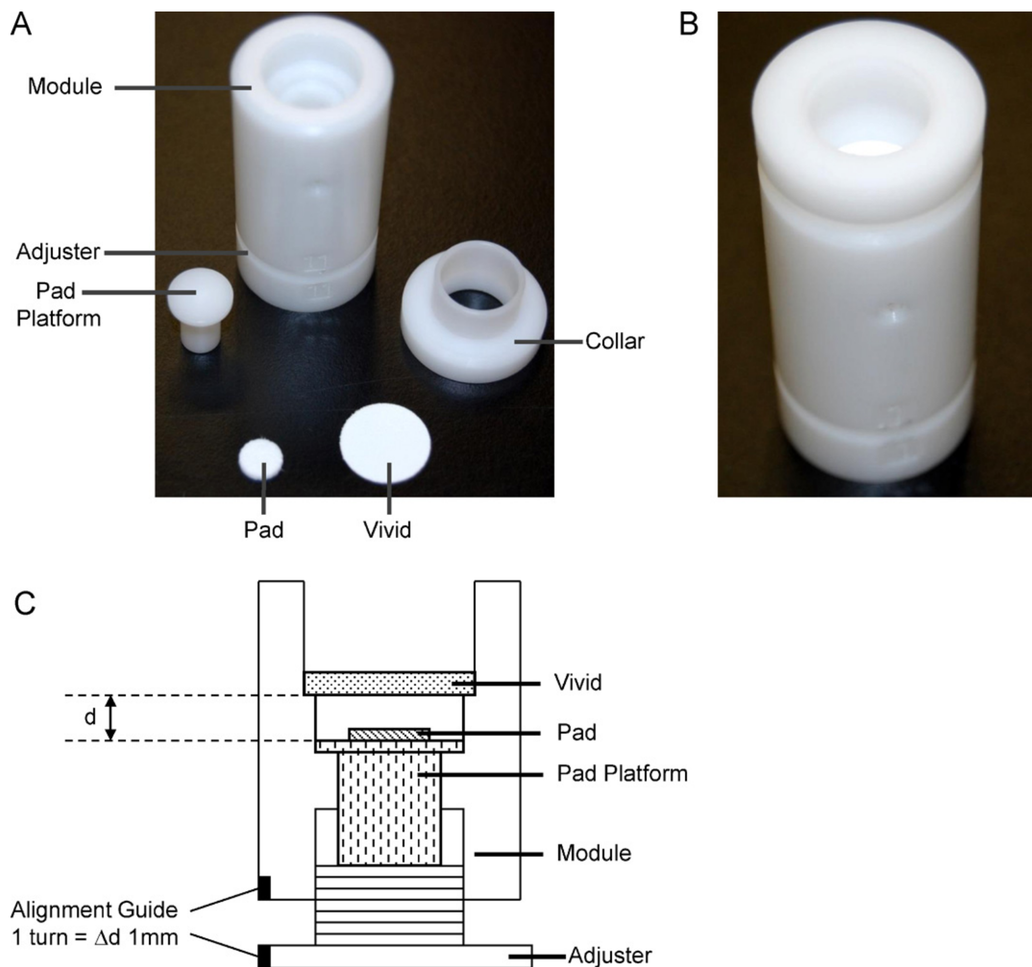

Fig. 1. Plasma separation module. (A) Exploded image of components comprising the module. The module serves as the base assembly while the adjuster moves up the pad platform through a threaded mechanism for precise positioning of the plasma collection pad with respect to the Vivid membrane. (B) Image of the assembled module ready for plasma separation. (C) Schematic diagram showing a cut-away view of the module. An anti-rotation insert is used for raising the plasma pad platform. The alignment guide allows positioning of the platform with respect to the lip of the module that holds in place the Vivid membrane with the ring collar shown in (B).

insert was designed as illustrated in Fig. 1C. This device allowed the evaluation of different materials for selection (see Section 3.2) as well as optimization of their use.

In order to develop a filter-based plasma separation device, two distinct components are necessary: a blood cell separation membrane and a plasma collection pad. For the blood cell separator membrane, Pall Corporation's Vivid membrane was selected that utilizes increasing capillary number (decreasing pore size) to achieve blood cell separation. Based on the manufacturer's specifications, the Vivid membrane was sized to a disc of 15.6 mm diameter in order to allow the separation of 2 drops of blood which was estimated nominally to equal 75 μL. Ideal properties for the plasma collection pad include efficient collection of plasma from the separation filter, rapid collection of plasma and efficient elution of plasma with minimal to no loss of target analyte. To identify suitable collection membranes, candidate plasma collection pads were next screened for their ability to fulfill the above criteria. The various candidate collection membranes were accordingly sized to hold up to 30 μL of volume and laser cut into circular discs for pairing with the Vivid membrane.

3.2. Screening of plasma collection pads

As a model disease to test this system, the HIV infection state in infants was simulated. In babies born to infected mothers, maternal antibodies against HIV are transferred, rendering antibody detection tests ineffective. Thus the p24 core antigen has to be targeted for unequivocal identification of the disease state. Given the inheritance of maternal antibodies, p24 in the blood is immunocomplexed to maternal antibodies and these antigen–antibody complexes must be disrupted before the detection of p24 can occur. Experimentally, 100 pg/mL of p24 antigen was immunocomplexed using 10k-fold excess monoclonal antibodies targeting two distinct epitopes of the protein. This immunocomplexed antigen was spiked into fresh infant blood and a 75 μL volume of that antigen-spiked blood was applied to devices containing plasma collection pads composed of glass fiber, cellulose and polyester (Table 1). After blood separation was complete, the plasma collection pads were removed and subjected to a quantitated p24 assay utilizing a heat shock pre-treatment step for immune complex disruption (Parpia et al., 2010). Outputs were compared to a standard solution phase reaction using 25 μL of plasma derived from low-speed centrifugation of the antigen-spiked blood.

Separation times did not vary among the various collection pads used, indicating that the blood separation membrane is the limiting factor in determining the time for separation (Table 1). The end-point for blood separation was determined visually by the disappearance of blood from the surface of the Vivid membrane. Pads were weighed before and after separation to determine the volume of plasma collected by each pad. Of the plasma collection pad candidates tested, the material composition of the pad did not seem a factor in the amount of plasma collected as pads made of either glass

Table 1
Screen of initial plasma collection pads for volume of collection and elution of 100 pg/mL of immunocomplexed p24 antigen. Pads were assayed using a p24 test with heat shock and the signal outputs in the form of arbitrary absorbance units (A.A.U.) were compared to a solution reaction using 25 μL of centrifuged plasma containing the same concentration of immunocomplexed p24 antigen.

Manufacturer	Pad	Material	Diameter (in mm)	μL plasma collected	Time for separation (min)	100pg/mL p24 A.A.U.
Ahlstrom	111	Glass	11	28	3.5	14.1
	121	Glass	8	4	4.0	2.3
	142	Glass	6	30	3.5	14.8
	151	Glass	9	28	3.5	5.5
Whatman	903	Cellulose	11	27	3.5	8.1
	Fusion 5	Proprietary	9.5	29	3.0	17.8
Pall	Accuwik	Polyester	8	27	3.5	20.1
	Leukosorb B	Proprietary	12	31	3.0	22.9
	Type III	Cellulose	11.2	29	3.0	15.7
	A/B	Glass	7	25	4.0	11.0
	A/D	Glass	7.5	28	3.5	25.8
	A/E	Glass	11	24	4.5	13.0
–	Solution Rxn	–	–	–	–	25.6

fiber, cellulose or polyester all collected ≥25 μL of plasma (Table 1). The pads that collected ≥25 μL of plasma were then assayed to evaluate their ability to tolerate heat shock for immune complex disruption and elute p24 protein following complex dissociation (Table 1). More than one pad performed well in elution of p24 compared to the solution phase reactions using of 25 μL of centrifuged plasma. Based on this initial screen, 5 pads were selected with the highest p24 signals and evaluated further for their assay performance.

3.3. Selection of plasma collection pad

In the next round of testing, multiple replicates were performed of the above experiment and included additional controls such as a blood sample containing 50 pg/mL immunocomplexed p24 antigen (which represents the limit of detection of the assay) and blood samples with no p24 to assess assay background contributions (and not assay specificity which was validated separately). On average, all of the candidates performed similarly when used in the assay. However one pad, the A/D, yielded lower background than the other pads and similar p24 signal values to comparable solution phase reactions (Fig. 2). Also given its more compact diameter of 7.5 mm, which enables greater flexibility in physical manipulations during the assay process, this pad was selected for the plasma collection component of our device.

3.4. Robustness of plasma separation method

To test the consistency and robustness of the device, 25 fresh infant whole blood samples were obtained with varying hematocrit (Ht) values. 75 μL of each infant sample was applied to the prototype plasma separation modules and both the times taken for plasma collection and the volumes of plasma collected were measured. Table 2 summarizes the results. The average time for plasma collection was 4.3 ± 0.8 min and the volume of collection was 27.4 ± 2.1 μL, with Ht values ranging from 21 to 48% (Avg. Ht = 34.0 ± 6.7%). An additional 5 fresh infant whole blood samples were obtained subsequently and spiked with 50 pg/mL immunocomplexed p24 antigen and 100k copies/mL of HIV. Separation was performed and total p24 signal outputs compared to solution phase reactions using 25 μL of plasma taken after centrifugation of the spiked blood samples (Fig. 3A). In all five samples, the results of the device-separated plasma reactions were equivalent to the centrifuged plasma reactions. With these evaluations, it was confirmed that this device is capable of blood separation, metered plasma collection and efficient elution of p24 antigen from the collected plasma.

In certain point-of-care settings where a device such as this would be deployed, there might be user interruptions in between the blood separation step and its transfer to the diagnostic assay due to patient flow and limited availability of test operators. Thus a test was performed to see if the recovery of p24 antigen

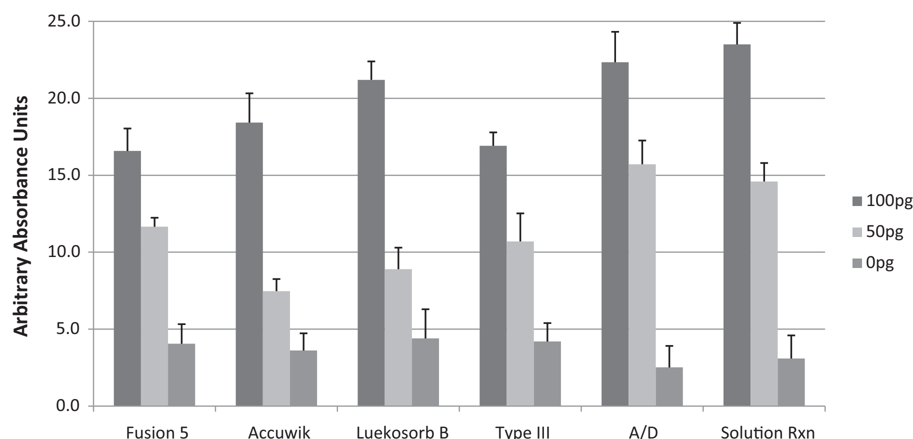

Fig. 2. Dose–response of candidate pads. Plasma collection pads selected from the initial screen presented in Table 1 were subjected to a dose–response of immunocomplexed p24 spike into normal whole blood. Performance of the pads is compared to equivalent solution phase reactions using 25 μL of centrifuged plasma for each sample dilution. Error bars represent 1 standard deviation of two replicates per sample.

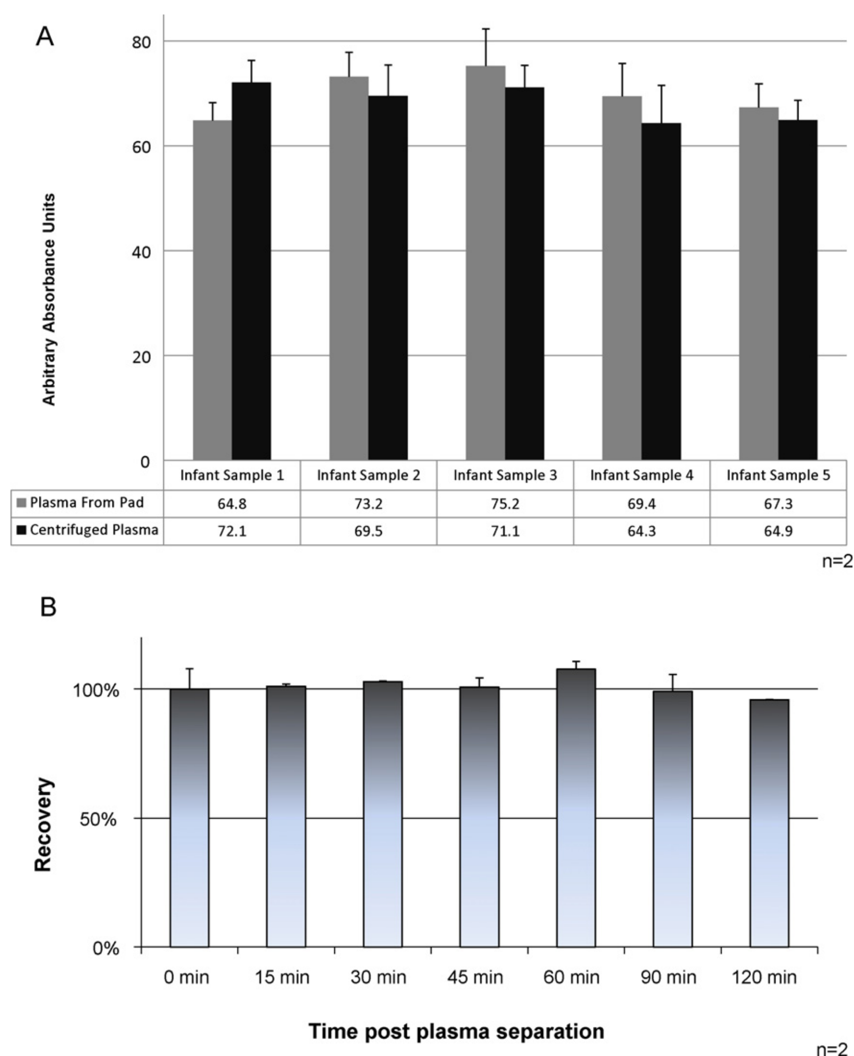

Fig. 3. Robustness of separation method. (A) Graph of p24 absorbance signals from 5 infant samples spiked in with 50 pg/mL recombinant p24 and 100k copies/mL whole virus and processed either with centrifugation for obtaining fractionated plasma or use of the plasma separation device for the collection of plasma in the A/D pad. (B) Recovery of immunocomplexed p24 antigen is graphically represented for A/D pad samples processed immediately after separation and at the time points noted up to 120 min post-separation. Error bars in (A) represent 1 standard deviation of two replicates per infant sample and in (B) two replicates per time point.

collected in a pad is affected for up to 2 h post-plasma separation at a nominal room temperature of 25 °C (Fig. 3B). Immunocomplexed p24 antigen was spiked into fresh blood at a final concentration of 100 pg/mL. 75 μL of this spiked blood was applied to plasma separation modules. The pads were removed and processed at various times post-separation. Fig. 3B shows the results from that experiment. Essentially, there was no loss of signal observed whether the pad was removed and processed immediately after separation or at time intervals up to 2 h post-separation. This indicates that the pad with collected plasma containing analyte can be left in the device for up to 2 h at room temperature, essentially serving as a protective environment for the separated plasma.

4. Discussion

The need for point-of-care diagnostics is highlighted by the growth of infectious disease and other pathogen related infections in both the developed and developing world. For many diagnostic tests, the test matrix is plasma or serum separated from whole blood, the source of that whole blood being a finger/heel stick or venipuncture. Ideally, the whole blood sample would be rapidly separated and the plasma eluted efficiently using a device with no moving parts, a small footprint and is readily disposable. These requirements are particularly pronounced in the developing world where access to capital equipment like centrifuge machines and a reliable power supply are limited resources. This report describes the development of a prototype device utilizing vertical flow of blood samples, a blood filtration membrane as well as a plasma collection pad that can fulfill the specifications for rapid, simple to operate and efficient plasma separation.

HIV infection is a major cause of death among infants in developing countries. In 2008, there were approximately 430,000 new infections in children worldwide (WHO, 2008). The mortality rate among HIV-infected infants is as high as 45% by the first year of age and 59% by the second year (Dabis and Ekpini, 2002). The initiation of anti-HIV treatment before 12 weeks of age could reduce the rate of infant mortality by 75% (Violari et al., 2008). Thus, the early diagnosis of HIV infection with timely treatment initiation has the potential to drastically reduce the rate of infant mortality from HIV infection. Recently the development and validation of a lateral flow HIV p24 antigen assay similar to antibody tests used commonly to diagnose HIV infection in adults were described (Parpia et al., 2010). Such an assay has the chance for the highest global health impact, particularly in resource-limited countries suffering the greatest burden of disease.

Table 2
Separation evaluation of infant whole blood using the plasma separation module.

Sample #	μL plasma collected	Time for separation (min)	Hematocrit
1	26.1	3	31.1%
2	28.7	4.5	30.9%
3	30.6	5	36.3%
4	30.2	5	45.6%
5	30.0	4.5	37.4%
6	26.2	5	29.0%
7	28.1	5	39.5%
8	26.8	4	31.2%
9	24.4	3.5	32.5%
10	26.1	4	32.1%
11	30.2	3	40.8%
12	27.9	2	27.3%
13	23.4	5	33.8%
14	25.1	4.5	29.7%
15	27.3	5	37.4%
16	25.4	4.5	36.8%
17	29.2	4	36.0%
18	28.0	5	48.1%
19	29.9	4.5	25.4%
20	26.0	5	43.8%
21	25.1	5	40.2%
22	26.5	3.5	20.8%
23	25.7	4.5	31.4%
24	27.4	4.5	24.6%
25	29.8	4	28.2%
AVG	27.4	4.3	34.0%
STD	2.1	0.8	6.7%

The ongoing development of the assay into a point-of-care device for field-deployable pediatric HIV diagnosis requires a low-cost, rapid and efficient blood separation module as part of the test. In this study, a prototype blood separation device made of machined plastic parts was produced. Next off-the-shelf commercial components were tested and selected that allow separation of small volumes of fresh blood (75 μL) in 3–5 min, yielding over 65% of the available plasma and eluting 100% of the input p24 antigen whether the source was intact HIV or recombinant p24 antigen. An additional requirement for the detection of p24 in the pediatric context is the need to perform heat shock on the collected plasma in order to liberate immunocomplexed antigen. This method of plasma collection into a pad is compatible with heat shock, releasing immunocomplexed antigen for efficient elution and detection by a lateral flow test strip. In this report it was showed that the results obtained with the pad-based collection methods were equivalent to centrifuge-derived plasma. While in this study fresh infant heel stick blood was used, successful testing of the device was achieved with adult blood collected freshly into 80 μL capillary tubes as well as venipuncture blood collected into EDTA, citrate and heparin coated tubes (data not shown).

In order for this prototype device to be evaluated fully additional work is necessary. In particular, (a) the development of molded parts for reduced size and operator friendly use of the plasma separation device, and (b) field use of the plasma separation device on target populations and incorporation with a diagnostic assay such as our pediatric HIV test. Work is already underway addressing these issues, particularly the development of molded parts for simple sonic welding of the membranes to a low-cost injected-molded plastic frame. Given the availability and scale of the materials used for this device, is anticipated to cost of one-half USD $1 or less.

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